A LABORATORY MANUAL

A LABORATORY MANUAL OF PHYSIOLOGICAL AND CLINICAL CHEMISTRY AND TOXICOLOGY

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Preface.

To offer to the medical profession in general and to the medical student in particular a new work embodying laboratory methods of clinical chemistry and toxicology, where so many admirable treatises are in existence, may appear at first sight an unprofitable and useless labor. But all such previous works have either been too voluminous for general use, or too complicated to serve as an ordinary working manual. What the student needs is a work brief enough to give him a clear understanding of the methods of chemical diagnosis, and broad enough so that its pages may comprise all that he will need in the intelligent practice of his profession. While this little work does not pretend to be a complete working manual of medical chemistry and toxicology, if it accomplishes the purpose for which it was written, that of leading the

student by simple steps to the more complicated methods of clinical chemical diagnosis, the authors will be thankful that their labors have not been in vain. Our thanks are due to Dr. Thorpe for his many useful suggestions and kind assistance while in preparation of this manual.

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PART I.

PHYSIOLOGICAL CHEMISTRY.

I.

Egg Albumen.

Take 20 c.c. egg albumen (white of egg) and shake well in a flask with 150 c.c. of water. Filter, and the filtrate, apart from a slight opalescence, should be wholly clear. With the filtered fluid try the following tests:

1. Heat a portion in a test-tube to boiling, and there is a white cloudiness, but a separation of coagulated albumen does not take place. This follows by the careful addition of dilute acetic acid (10%), drop by drop. The reaction of all albumens must be acid for coagulation.

2. To a portion in a test-tube add one-half the amount of concentrated salt solution and divide into two parts. Heat one part to boiling by itself, and there occurs a flocculent pp. or coagulation. To the other part add acetic acid until slightly acid, and it becomes cloudy in the cold, but gives on heating a flocculent pp. The greater amount of salt an albumen solution contains, so much more independent of the acidity is the coagulation by heating.

3. To a portion in a test-tube add glacial acetic acid, and heat. Acid albumen is formed. Now cool the tube and add NaOH, and a pp. is formed, while the reaction is still slightly acid; but if NaOH be added in excess, the pp. is dissolved and alkali albumen is formed.

4. Heat a portion with half the amount of NaOH, and there is formed alkali albumen. Cool and add dilute H_2SO_4 — the albumen is pp., but again dissolved in an excess of the reagent.

5. To a portion in a test-tube add $CuSO_4$, and there arises a bluish-white pp. of copper albuminate, which, on the addition of NaOH, is dissolved in a deep-blue fluid. The salts of the other heavy metals in general give pps.

6. To a portion in a test-tube add $HgCl_2$. There arises a thick, white pp. which is insoluble in excess of the reagent, but soluble in conc. salt solution.

7. Heat a sample with strong NaOH and 3 drops of neutral lead acetate. The solution is blackened, due to the separation of sulphur. Upon acidifying with HCl, there is a separation of large black flakes, while in serum albumen there is only a cloudy gray fluid. This depends upon the fact that the sulphur in egg albumen is separated in larger quantities, and

the lead albuminate is less soluble in HCl than that of serum albumen.

8. Shake a portion with ether, and there is a complete coagulation.

9. To a small portion add conc. HNO_3, and there arises a pp. which is not dissolved even when the amount of acid equals half the amount of albumen solution. To this end it is necessary to add much larger quantities or to warm.

Color Tests for Albumen.

10. Biuret Test. — To a portion in a test-tube add an equal amount of NaOH, and then a few drops of a very dilute solution $CuSO_4$. (One drop $CuSO_4$ to a test-tube of water.) Purple to amethyst color.

11. Millon's Test. — To a portion add a few drops of Millon's reagent, and boil. Brick-red pp.

12. Adamkiewicz Test. — Take one part conc. H_2SO_4 and two parts of acetic acid. Add the albumen solution carefully without shaking, so that it shall overlay the acids. Warm slightly, and at the point of contact there develops a beautiful purple-red zone, which grows more intense in standing.

13. Xantho Proteic Reaction. — To a portion add strong HNO_3 until the pp. which is

formed no longer dissolves. Heat, and the pp. becomes colored yellow. Now cool and add NaOH in excess — color changes to orange.

Now take 1 part of the albumen solution and nine of water and perform all of these tests again. Note particularly the action with Cu (used in sugar tests) — with Hg (used in urea test) — acetic acid (used in mucus test) — and with Pb (action similar to that of bismuth, also used in sugar test).

II.

Nucleo-Proteids.

Take ½ cake of yeast, place in a mortar, add 50 c.c. of water and 3 to 5 drops of a very dilute solution NaOH (until neutral or slightly alkaline). Rub well with a pestle, filter, and with the filtrate perform the following tests:

1. Heat a small portion to boiling in a test-tube. It should coagulate. How does it differ from egg albumen?

2. Add to a portion, in a test-tube, dilute (10%) acetic acid. There arises a pp. which is soluble in excess of the reagent.

3. Take 25 c.c. of the solution and add $AgNO_3$ until all the NaCl is precipitated. Filter,

pass H_2S through the filtrate until all the silver is precipitated as sulphide of silver. Warm and filter again (filter more than once if necessary, in order to get a clear filtrate).

Take 10 c.c. of this solution and add an equal amount of dilute H_2SO_4, and warm for some time (at least 20 minutes) upon the water-bath. Cool, alkalinize with NH_4OH (there must be an excess), and filter. To the filtrate add silver nitrate — and there arises a white pp. of xanthine bodies.

4. Take 10 c.c. of the solution, add 2 c.c. of conc. HCl, and cook for some time upon the water-bath, replacing the water as it evaporates. Then cool, neutralize with NaOH, add a few drops of $CuSo_4$, and heat again to boiling. There arises a separation of red or yellow suboxide of copper, which is best seen upon plunging the tube into cold water. This is a reducing body, but it is probably not sugar.

5. With a small portion try the biuret reaction. Purple to amethyst color.

6. Try Millon's reagent. Brick-red pp.

7. Add alcohol to a portion and note the effect. Coagulation?

8. Saturate a portion with $MgSO_4$ in substance, warming slightly to effect complete solution. Note effect. Precipitation?

9. Take two yeast-cakes, make a solution of them in the same way as given above, and precipitate the nucleo-proteid by warming and the careful addition of dilute acetic acid. Decant off the fluid, filter, and wash the pp. with water, alcohol, and ether. After thoroughly drying, rub up the pp. with 4 grammes saltpeter mixture,[1] heat to melting in a crucible, dissolve the residue after cooling in nitric acid, and heat until the resulting nitrous acid fumes have been driven off. Take 10 c.c. of ammonium molybdate solution, and add to it the nitric acid solution drop by drop. A yellow pp. shows the presence of phosphorus in the form of the acid. To another portion of the solution add NH_4OH in excess; it should remain clear. Now acidify slightly with acetic acid and add uranyl nitrate solution. There is formed a yellowish-white pp. if phosphoric acid is present.

Nucleo-Albumen.

Make a solution of casein (2 grammes of casein to 100 c.c. of water) and perform the same tests as for nucleo-proteids. Note whether nucleo-albumens contain —

(1) Xanthine Bodies.

[1] This mixture is composed of 3 parts KNO_3 and 1 part Na_2CO_3.

(2) A Reducing Body.
(3) Phosphorus.

III.

Albuminoides.

Take 5 grammes gelatin, add to it 100 c.c. water, and warm. It dissolves, but upon cooling it becomes semisolid, thus behaving directly opposite to albumen, which coagulates by heat. With the warm solution perform the following tests:

1. To a portion add a few drops of Millon's reagent, and boil. Brick-red pp. This is due to the gelatin containing albumen as an impurity, as pure gelatin does not respond to Millon's test.

2. To another portion add an equal amount of NaOH and a few drops of weak copper sulphate solution. Biuret reaction. Amethyst color.

3. Add nitric acid, and warm. Yellow color. Cool, and alkalinize with NaOH. Orange color. Xanthoproteic reaction.

4. Cook a portion with NaOH and a few drops of subacetate of lead. Although gelatin

contains sulphur, there is no blackening, as the sulphur is less easily split off than in egg albumen.

5. To a portion in 4 test-tubes add respectively:

(*a.*) Nitric acid. No pp.

(*b.*) Potassium ferrocyanide. No pp.

(*c.*) Acetic acid. No pp.

(*d.*) Copper sulphate. No pp.

6. To a portion add some mercuric chloride. There is no pp. formed. Now add a few drops of HCl, and there is formed a pp. which is soluble in an excess of the acid.

7. To a portion add some tannic acid. There is no pp. Now add some NaCl, and there is formed a copious pp.

8. Try the Adamkiewicz reaction. A negative result with pure gelatin.

IV.

Blood-Serum.

Blood-serum, as well as serous exudations, contains an albumen soluble in water — the serum albumen; and one which is insoluble in water — the serum globulin. This latter is

held in solution by the alkaline reaction and the salts of the serum.

Rub 20 c.c. of blood-serum in a mortar with an excess of ammonium sulphate (about 15 grammes) for a long time, in order that the fluid may be surely saturated with the salt. By this means both albumens are precipitated. Filter through a dry filter, and the filtrate is albumen free. Heated to boiling and treated with acetic acid, it remains clear. Now wash the pp. with a half-saturated solution of ammonium sulphate, and the albumen in part becomes redissolved, while the globulin remaining upon the filter can easily be dissolved by water, with the aid of the salt mixed with it.

1. Solution of Globulin.—Add a few drops to half a test-tube of water. Precipitate. Solubility in water?

2. Add a mere trace of very dilute NaOH to the previous pp. in the test-tube, and it becomes redissolved.

3. Neutralize carefully with dilute HCl, and the globulin becomes again precipitated. If in (2) too much NaOH were added in proportion to the amount of globulin, it is not reprecipitated, for the NaCl formed holds it in solution.

To these respective solutions, (a) serum

albumen and (b) serum globulin, add the reagents employed for egg albumen. Note how serum albumen and serum globulin differ from each other, and also how they both differ from egg albumen.

Now take 10 c.c. of blood serum, add water to 100 c.c., and perform the following tests:

1. Heat to boiling — no change. Add a drop or two of HNO_3 and heat again, and there is a separation of coagulated albumen.

2. Add acetic acid and potassium ferrocyanide. Cloudiness — then a pp.

3. Acidify with HCl and then add phosphotungstic acid. Copious gelatinous pp.

4. Add tannic acid. Copious pp.

5. Add mercuric chloride. A pp. — which is soluble in NaCl solution.

6. Add a few drops of Millon's reagent, and boil. Reddish-brown pp.

V.

Albumoses.

Use solution provided — 2 grammes to 100 c.c. of water.

1. Heat a portion to boiling; remains unchanged also after adding acetic acid (a drop) and NaCl.

2. Acidify with acetic acid and add conc. salt solution. The solution becomes cloudy, but upon heating again becomes clear. Upon cooling — cloudy.

3. Add a few drops HNO_3, and there is a cloudiness to a pp. which is soluble in excess. By warming and NaOH — xanthoproteic reaction.

4. Add a few drops acetic acid and the potassium ferrocyanide. Cloudiness, which disappears on heating (not always wholly).

5. Biuret test — rose to purple color.

6. Perform the tests (3) — (4) — (5) — (6) — of blood-serum. Insoluble precipitates.

VI.

Sugars.

1. Take a small portion of milk-sugar and heat it to melting upon platinum foil or a piece of tin plate. There is developed an odor of caramel and a carbonizing, with the final leaving of a slight amount of ash.

Make a solution of 2 grammes milk-sugar to 100 c.c. water, and perform the following tests:

2. Trommer's Test. — Add to a few c.c. of the solution an equal amount of NaOH, and, drop by drop, $CuSO_4$, shaking constantly. There arises a deep-blue solution, which upon heating gives a separation of red or yellow suboxide of copper.

3. Moore's Test.— Add to a portion an equal amount of NaOH and heat to boiling; yellow to brown color, with the odor of caramel, especially after acidification with dilute H_2SO_4.

4. Nylander's Test. — Saturate a portion with sodium carbonate in substance, and boil. While boiling add a very small portion of bismuth subnitrate and keep for some time at a boiling point. Gray to black color, with the separation of fine metallic bismuth.

5. Add some ammonia and a few drops of $AgNO_3$, and heat. Separation of metallic silver in the form of a glistening mirror upon the test-tube. The mirror is especially marked if a drop of NaOH be added before boiling. As the $AgNO_3$ in this test is changed to fulminating silver, care must be taken against its possible explosion by afterwards filling the test-tube with water.

6. Indigo Test. — Add indigo carmine to the sugar solution until distinctly blue, and then add sodium carbonate solution until alkaline,

and warm. The solution becomes first violet, then red, yellow, and finally colorless. Pour half of the solution in another test-tube and shake with air. It again becomes blue, but upon heating is again decolorized. This continues until all the sugar is used up by oxidation.

7. Phenylhydrazin Test.—Take 25 c.c. of the solution in a small beaker, and add 1 gramme phenylhydrazin hydrochlorate and 2 grammes sodium acetate. Place in boiling water for 30 minutes, and then cool by plunging the beaker in cold water. A yellow crystalline deposit is thrown down, which, when examined under the microscope, is found to consist of wide prisms of phenyl-lactosazon.

Now make a 2% solution of grape-sugar and perform the same tests.

8. Fill two fermentation tubes with solutions of grape and milk sugar respectively. Shake with a piece of fresh yeast until disintegrated, and place in a brood oven until the next day. Note the difference. Milk-sugar ferments slowly and with difficulty.

9. Grape-sugar can be dissolved in conc. H_2SO without color; milk-sugar is colored brown when dissolved in the same acid.

10. Phenylhydrazin Test. — Bright-yellow

needles of phenyl-glucosazon, arranged in bundles or sheaves.

11. In two test-tubes place a few c.c. of grape and milk sugar respectively, and add one or two drops of a 15% alcoholic solution of alpha-naphthol. Then let about 1 c.c. of conc. H_2SO_4 run carefully down the side of the test-tube so as to underlie the other fluid: there is formed at the junction of the two fluids a beautiful violet-red ring that increases intensity upon slight shaking.

Make a 2% solution of cane-sugar and try the reducing tests (copper and bismuth). Cane-sugar will not reduce.

Try the fermentation test with yeast, the same as for grape and milk sugar. After fermentation again try the reducing tests. The cane-sugar is a disaccharide, and, by the action of the invertion of the yeast, has been split up into its two mono-saccharides, glucose and levulose, both of which will reduce.

Cane-sugar does not form a compound with phenylhydrazin.

Starches.

Measure out 100 c.c. water, and with a portion of the same rub up 1 gramme of starch in a mortar. Pour into an evaporating dish

and with the rest of the water wash all the starch into the dish. Now heat to boiling under constant stirring. Allow to cool and perform the following tests:

1. Add to a portion in a test-tube 3 or 4 drops of a solution of iodine and potassium iodide. Blue color.

2. Boil a portion for 20 minutes with dilute HCl. Cool, and to a portion add iodine solution again. Mahogany-red color due to erythrodextrin. The starch has been converted to erythrodextrin and glucose (grape-sugar). Alkalinize another portion fully, and test for glucose with reducing tests.

3. Take 10 c.c. of the starch solution, add 1 c.c. saliva, and place in a brood oven until the next day. Test for erythrodextrin and maltose.

VII.

Fats.

1. Rub a small portion of lard or oil upon paper (not filter-paper), and it becomes translucent.

2. Dissolve a piece of lard the size of a pea in a few c.c. of alcohol with the aid of a little

ether, and add a couple drops rosolic acid, which gives the solution a yellow color. Now add from a burette a $\frac{1}{10}$ Normal sol. NaOH, and one or two drops changes the color to red.

3. Rub up a small portion in a mortar with bisulphate of potash, and heat the mixture in a dry test-tube. Visible fumes and the pungent odor of acrolein show a neutral fat.

4. Warm a small portion in a test-tube with sodium carbonate solution. The fat is distributed in the solution, but the latter is not clear; saponification does not take place.

5. Dissolve 5 grammes KOH in 35 c.c. alcohol, by warming over the water bath. Then melt 5 grammes of lard over the water bath, and add to it while stirring the KOH solution. Cook for 20 to 30 minutes, or until a small portion in a test-tube of water shows no oil drops. There is complete saponification.

6. Add to 65 c.c. water 20 c.c. dil. H_2SO_4 (20%) and warm somewhat, but not to boiling. Now add the soap solution, while still hot, carefully to the dilute acid (there is a liability to snap). Allow to cool, and the fatty acids arise to the surface of the fluid as a layer which can be broken through, and the watery solution which contains the glycerine can be poured off. Hot water should be added

to this fat, and the process repeated until the wash water is no longer acid. Dry the fatty acids between filter-paper.

Fatty Acids.

1. Behavior to paper same as neutral fat.

2. Make a solution with alcohol and ether, and add NaOH as above (2), using rosolic acid as an indicator. It takes 2 or more c.c. to change the color to red. They are acid.

3. No acrolein by heating with bisulphate of potash.

4. Add to a portion a half-saturated solution of sodium carbonate, and heat. The fatty acids dissolve with the development of CO_2 and the formation of a sodium soap. Dip the test-tube in water, and the soap solidifies.

5. Dissolve 2 grammes of fatty acids in 100 c.c. of water, with the addition of the smallest possible quantity of NaOH needed to effect solution. With this solution perform the following tests:

(*a.*) Add HCl. Separation of fatty acids.

(*b.*) Addition of chloride of calcium. Insoluble chalk soap. The fluid no longer foams upon shaking.

(*c.*) Add lead acetate, and warm. A white

pp. which upon warming becomes tough and crumbly — "Lead Plaster."

(*d.*) Take a little of the solution, add a few drops of oil, and shake. There is formed a fine white emulsion.

(*e.*) Try the same thing with sodium carbonate solution and oil. Negative result. An emulsion can only be formed when some free fatty acids are present, as when a fat becomes rancid.

(*f.*) Place some of the fat in a test-tube and an equal amount of the fatty acids in another. Place both tubes in a beaker of water, and heat upon a piece of wire gauze, occasionally stirring the water so that the temperature shall be the same everywhere. It will be noticed that the fat melts much sooner than the fatty acids obtained from the same fat.

Glycerine.

Take some of the watery solution from Fats (6), and with it perform —

1. Trommer's Test. — Blue solution, but no reduction of copper.

2. Mix some glycerine with a little borax and heat a small portion in the flame upon platinum foil. The flame is temporarily colored green.

3. Try the acrolein test.

4. Make an emulsion with oil and soap solution, and examine under the microscope.

VIII.

Chemistry of Blood.

1. Take a piece of lacmoid paper, wet with conc. salt solution, and place upon it with a glass rod a drop of blood. A blue spot shows the alkalinity of blood.

2. Add to about 10 c.c. water a few drops of blood, shaking thoroughly. Then add some fresh tincture guaiac (made by dissolving a penknife-pointful of the powder with some alcohol in a test-tube) until the solution becomes milky. Now add some old oil of turpentine, and upon shaking the solution becomes colored an intense blue.

3. Add to a few c.c. blood several times its volume of peroxide of hydrogen. There arises a strong foaming and development of oxygen, with gradual decomposition of the blood-coloring substance. This catalytic action of the blood is lost in the presence of hydrocyanic acid, but the acid must be added to the blood

directly. In the blood of an animal killed by hydrocyanic acid, this catalytic action persists.

4. Add to a little blood in a test-tube some ether and water, and shake thoroughly. The coloring matter of the corpuscles becomes dissolved, and the blood becomes "lakey." The biliary acids have the same action upon blood.

5. Dilute 10 c.c. blood to 100 c.c. with water and perform the following tests:

(*a.*) Take a half test-tube of blood, place in the spectroscope, and continue to add water until the two lines of oxyhæmoglobin in green and yellow can be seen. Now add a few drops of ammonium sulphide, and allow to stand for a few minutes. There is a change of color from red to green, and the appearance of only one line in the spectroscope, that of reduced hæmoglobin, occupying a position midway between that of the other two.

(*b.*) Take another portion of the blood solution and add a few drops of freshly prepared conc. solution of potassium ferricyanide. The solution becomes brown and shows the characteristic four-line spectrum of methæmoglobin. Now add a few drops of ammonium sulphide, allow to stand for a short time, shake strongly, and again examine with a spectroscope. The spectrum of oxyhæmoglobin will be found.

Methæmoglobin can be reduced to hæmoglobin, and again oxydized to oxyhæmoglobin.

(*c.*) Pass hydrogen sulphide through some of the dilute blood and examine with a spectroscope — the spectrum of sulph-hæmoglobin. It resembles that of methæmoglobin except that the line in red is farther to the left, and that it cannot be reduced. Try the reduction test, same as in (*b*).

(*d.*) Add to 10 c.c. of the dilute blood 1 c.c. NaOH, and heat. The solution becomes brownish-green. Examination with the spectroscope shows one broad band of absorption extending to red — the spectrum of hæmatin in alkaline solution. Now add a few drops of ammonium sulphide, allow to stand for a short time, and again examine with a spectroscope. The one absorption band has disappeared, and in its place will be found the two lines of reduced hæmatin.

6. Pass ordinary illuminating gas through 50 c.c. blood until it becomes a cherry-red. Examine with the spectroscope. The spectrum resembles that of oxyhæmoglobin, except that it cannot be reduced. Try the reducing tests.

(*a.*) Add to this blood and to genuine blood equal amounts (about $\frac{1}{2}$ its volume) of NaOH. Note the difference; place a drop of

each in an evaporating dish, and note difference in color.

(*b.*) Add to 10 c.c. carbon monoxide blood 15 c.c. of a 20% solution potassium ferricyanide and 2 c.c. acetic acid — and to the same amount of unmodified blood add the same reagents. Allow to stand a few moments, and note the difference.

(*c.*) To 5 c.c. of carbon monoxide blood add four times as much water. To a measured portion of this add three times its volume of a 1% tannic solution. Do the same with unmodified blood, and note the difference.

7. Dry a drop or two of blood upon a microscope slide, and rub up the dried blood with the smallest possible quantity of sodium chloride. Place a cover glass upon it, and add a couple drops of glacial acetic acid to the edge of the cover glass, allowing the acid to flow under the latter. Now warm upon the water bath until evaporated, add a drop or two more of the acid, and allow to cool. Examine with a microscope ($\frac{1}{7}''$ or $\frac{1}{8}''$ objective). Small dark-brown, rhombic crystals of hæmin chloride show the presence of blood. Stains upon linen or wood can be soaked out in a normal salt solution and tested in the same way. These crystals can be obtained from

mere traces of blood, and their presence is conclusive of the presence of blood in medico-legal examinations.

8. Take 30 c.c. fresh blood, add 8 times as much water, and heat to boiling, with constant stirring. While boiling add carefully dilute (10%) acetic acid until slightly acid, and filter. The filtrate must be clear. Evaporate the filtrate to a small volume, and divide into two halves. With one, try Trommer's Test for sugar. The other half is to be still further concentrated, and a drop allowed to evaporate upon an object glass, and examined with a microscope. Crystals of NaCl.

The rest is to be evaporated to dryness in an evaporating dish, heated to redness, cooled, and dissolved in a little water. Examine for sodium chloride with $AgNO_3$ — for phosphates with ammonium molybdate.

If, in the above, after heating to redness, a black residue remains, add a little saltpetre mixture, and heat to redness again. After cooling, dissolve the residue, which should be colorless, in nitric acid, evaporate off the excess of the acid, and test for phosphates and chlorides as above.

9. Dry the pp. formed in (8), take 1 gramme of the dried pp. and rub thoroughly in a mortar

with 4 grammes saltpeter mixture. Heat to melting in a crucible for about 20 minutes, and after cooling dissolve the residue in dilute sulphuric acid (8 parts by weight conc. $H_2SO_4 + 3$ parts by weight of water). Filter, and to a portion of the filtrate, which should be clear, add some potassium sulphocyanide. A blood-red color shows iron.

IX.

Clinical Analysis of Blood.

Specific Gravity (Roy's method). — A number of test-tubes are two-thirds filled with a mixture of glycerine and water in different proportions, so that the specific gravity in the tubes shall vary between 1030 and 1070. Blood is then drawn from the tip of the finger or the lobe of the ear into a capillary tube connected with an ordinary hypodermic syringe. A drop of blood is placed in each tube, in which it will sink as long as the specific gravity of the glycerine mixture is lower than that of the blood, while it will remain suspended in a mixture the specific gravity of which is equivalent to its own.

Normal specific gravity — 1.046 — 1.067.

Alkalinity (Löwy's method). — Five c.c. of defibrinated blood, accurately measured, are mixed in a small flask with 45 c.c. water; if not defibrinated, the blood is mixed with the same amount of a 0.25% solution ammonium oxalate. Titrate the solution with a 1/25 normal solution of tartaric acid, until a drop placed upon a piece of lacmoid paper (which has been previously soaked in a conc. solution of magnesium sulphate) gives rise to a blue tinge.

Calculation — 1 c.c. $\frac{1}{25}$ normal solution tartaric acid = .0016 grammes NaOH.

Normal alkalinity in terms of sodium hydrate =182 to 218 milligrammes for every 100 c.c. of blood.

Quantitative Estimation of Hæmoglobin (Fleischl's method). — The instrument used is the hæmometer of Fleischl, and the principle of the method depends upon the comparison of the color of the blood, diluted with water, with that of a glass wedge stained with a red pigment. The principal part of the instrument consists of two semicircular compartments, one receiving light from a plaster-of-Paris reflector through the glass wedge and called the red chamber, the other receiving light directly from the reflector and called the white chamber.

To use the instrument, the two compartments are partially filled with water, and the required amount of blood is obtained by placing one end of the capillary pipette in contact with a drop of blood obtained from the tip of the finger or the lobe of the ear. The pipette is immersed in the white chamber and rotated between the fingers. By this method the blood passes into the water, and any traces of blood remaining in the pipette are carefully washed out. The two compartments are then completely filled with water, and the glass wedge so adjusted, by means of the screw, that the color in the two chambers shall be the same. The number facing the notch in the scale aperture of the platform will then indicate the percentage of hæmoglobin, that of a healthy individual corresponding to 100. As the normal amount of hæmoglobin contained in 100 grammes of blood is 13.7 grammes, the number 100 on the scale of Fleischl's instrument corresponds to 13.7%.

Calculate % according to equation:

100 : 13.7 : : reading on the scale : X

The Enumeration of the Red Blood Corpuscles (Thoma-Zeiss method). — The instrument used is the Thoma-Zeiss blood-counting apparatus.

Blood is drawn from the tip of the finger or lobe of the ear into the capillary pipette, to the mark 1 or 0.5, according to the degree of dilution desired. The blood is then diluted with Pacini's fluid, which is drawn into the pipette, past the bulb, until the mark 101 is reached. If the blood had previously been drawn to the mark 0.5, it now has been diluted 200 times; if to the mark 1, it has been diluted 100 times. The contents of the bulb are now thoroughly mixed by shaking. A drop of the mixture is placed in the counting-chamber, so that it shall completely fill it, and the cover-slip is adjusted. The slide is placed under the microscope, a $\frac{1}{7}''$ or $\frac{1}{8}''$ objective being used. Now focus until the small squares come distinctly into view. Each one of these small squares is $\frac{1}{20}$ m.m. long, $\frac{1}{20}$ m.m. wide, and $\frac{1}{10}$ m.m. deep; its cubic contents are therefore $\frac{1}{4000}$ cubic m.m. Count the corpuscles in at least 16 small squares and take the average, which will give the number of corpuscles in one small square, or in $\frac{1}{4000}$ cubic m.m. One cubic m.m. of this diluted blood will then contain 4,000 times the number contained in one small square, and one cubic m.m. of the undiluted blood will contain the product of this figure multiplied by the degree of the original dilution.

The Enumeration of the Red Blood Corpuscles by the Hæmatokrit. — The graduated glass tube is completely filled, by suction, with blood from the finger or ear. The blunt point of the tube is then quickly covered with the finger, and the tube fixed in the frame of the centrifuge. This is rotated for two to three minutes, and the number of corpuscles which have been thrown to the bottom of the tube are read off. Multiply the reading on the tube by 100,000, and the product is the number of red corpuscles in one cubic m.m.

The Enumeration of the White Blood Corpuscles (Thoma-Zeiss method). — This enumeration is done with the same apparatus as for the red corpuscles, except that a special diluting pipette is used. The diluting fluid is a 0.3% solution acetic acid colored with methyl-violet, which destroys the red corpuscles and brings out the white very distinctly. The pipette is filled with blood to the mark 0.5 if we wish to dilute 20 times, or to 1 if a dilution of 10 times is desired. Then fill the pipette to the mark 11 with the acetic acid solution. Place a drop in the Thoma-Zeiss cell, use a $\frac{1}{7}''$ or $\frac{1}{8}''$ objective, and focus as above. As the number of white corpuscles is smaller than the red, it is necessary to count

the number of corpuscles in a whole field; that is, in the area of one microscopic circle. Then determine the cubic contents of this microscopic circle, and from this calculate the entire number in one cubic m.m.

The cubic contents are determined as follows: Count the number of cross vertical lines in the field of the microscope, remembering that the distance between each two lines is $\frac{1}{20}$ m.m. This gives the diameter of the circle. Divide the diameter by 2, which gives the radius; square the radius, and multiply the square of the radius by 3.1416. The product is the area of the circle, and this area, multiplied by 0.1, which is the depth of the cell in a fraction of a millimeter, gives the cubic contents of the microscopic circle. The number of white corpuscles counted in this single field is therefore contained in the fraction of one cubic m.m. represented by the cubic contents of the field counted. From this calculate the number of white blood corpuscles in one cubic m.m. of diluted blood, and this, multiplied by the degree of dilution (10 or 20), will give the entire number in one cubic m.m.

Formula for the determination of the cubic contents of the circle:

$\text{Radius}^2 \times 3.1416 \times \frac{1}{10} =$ cubic contents of

X.

Saliva.

1. Add to some saliva in a test-tube a few drops of dilute (10%) acetic acid. Cloudiness due to mucin — not soluble in excess of the acid.

2. Add to saliva a few drops HNO_3. A cloudiness which becomes yellow upon heating. Cool and alkalinize with NaOH — orange color. This xanthoproteic reaction is due to mucin or an albuminoid body.

3. To a small portion of saliva add an equal amount of water and a few drops Millon's reagent. White pp., which becomes red upon warming. Due to mucin.

4. Add NaOH and a few drops of a very dilute $CuSO_4$ solution. Violet color due to mucin.

5. Add to a portion one drop of HCl and a few drops of a very dilute solution of ferric chloride, and shake thoroughly. Red color from the formation of ferric sulphocyanide. Saliva contains potassium sulphocyanide (KCNS), its presence being due to the gland containing adenin ($C_5H_5N_5$), which is a polymere of hydrocyanic acid (CHN).

6. Make a starch clyster (1 gramme starch to 100 c.c. water), and, after cooling, take 10 c.c. of this solution, add 1 c.c. of saliva, and digest at 40° C. for an hour. Then put into a fermentation tube and allow to stand until the next day. It will be found that the resulting substance (maltose) is fermentable.

7. Perform the same test as in (6), but add to the starch and saliva mixture 1 c.c. digestive HCl. The action of the ptyalin is annulled. Prove by Trommer's test and the fermentation tube.

8. Determine the reaction of saliva. It is normally alkaline, but in pathological conditions, as in fevers or diabetes, it is acid.

9. Examine some saliva under a microscope, and it will be found to contain certain morphological elements, such as salivary corpuscles, pavement epithelial cells, and various fungi and bacteria.

XI.

Chemistry of Gastric Juice.

Hydrochloric Acid. — 1. (*a.*) Add to some digestive hydrochloric acid in a test-tube a few drops of methyl-violet solution. Steel-blue color.

(*b.*) Make the same test with water. Violet color.

(*c.*) Make the same test with lactic acid. Violet color, with slight shade of blue.

2. Make the same test with HCl solution, diluted 1 to 5.

3. Take a portion of the HCl solution in 2 test-tubes. To one add an equal amount of water, to the other an equal amount of albumose-peptone solution; then add methyl-violet to both and compare the color.

4. Make the same tests with the HCl solution diluted 1 to 5. The presence of large amounts of peptone makes methyl-violet useless as a reagent.

5. Add to a portion of the lactic acid solution a few drops of methyl-violet, and divide into three parts. To No. 1 add an equal amount of water; to No. 2 add an equal amount 3% salt solution; to No. 3 add an equal amount conc. salt solution. Nos. 1 and 2 change color but slightly. No. 3, however, becomes a distinct steel-blue. Sodium chloride disturbs the reaction in the presence of lactic acid only when strongly concentrated, due to the separation of the HCl by the lactic acid.

6. Evaporate a few drops of the mixtures formed under 2, 3, 4, and 5, in a small evaporating dish, to dryness over a free flame, avoiding too strong a heat. Intense blue residue. Peptones disturb the reaction only when present in large quantities.

7. To a few drops of the digestive HCl in an evaporating dish add a few drops of tropæolin 00 solution, and warm gently. Lilac-blue color.

8. Add to a few drops of digestive HCl in an evaporating dish a drop of Günzburg's reagent, and evaporate to dryness over a free flame, avoiding too strong a heat. Beautiful purple-red residue. Make the same tests with 2, 3, 4, and 5. Peptone does not disturb the reaction and lactic acid does not give it.

9. To a little HCl in a test-tube add a drop of dimethyl-amido-azo-benzol. Brilliant red color.

10. Take two test-tubes, and in No. 1 place a few c.c. digestive HCl diluted 10 times; in the other place an equal amount of water. To each add a drop of dimethyl-amido-azo-benzol. No. 1 becomes a brilliant red; No. 2 becomes cloudy and yellow.

Lactic Acid. — The reagent used for the detection of lactic acid is Uffelmann's, made by

taking 10 c.c. of a 5% carbolic acid solution and adding a few drops ferric chloride. Amethyst-blue fluid.

1. To a portion of the reagent add a few drops digestive HCl. Decolorization.

2. To another portion add some lactic acid. Canary-yellow color.

3. To another portion add a mixture of equal parts of digestive HCl and lactic acid solution. Canary-yellow color. With Uffelmann's reagent lactic acid can be detected beside HCl, but not HCl beside lactic acid.

4. To Uffelmann's reagent add some lactic acid and place in 3 test-tubes. To No. 1 add an equal amount of water; to No. 2 an equal amount conc. NaCl solution; to No. 3 an equal amount 3% NaCl solution. Only No. 2 becomes decolorized. The presence of NaCl in small quantities does not disturb the detection of lactic acid.

Pepsin.—1. Place in 3 test-tubes thin slices of hard-boiled egg of equal size. To No. 1 add 10 c.c. digestive HCl; to No. 2 add 5 c.c. digestive HCl with pepsin; to No. 3 add 10 c.c. digestive HCl with pepsin. Place all three in the brood oven for an hour or more, and note the difference in the thinning of the slices, due to the digestive action of the three fluids.

2. Take the whites of 4 hard-boiled eggs, cut fine, and place in a flask, adding 500 c.c. of the digestive HCl with pepsin (1 gramme pepsin, 10 c.c. HCl, and water to 500 c.c.). Place in a brood oven for 2 to 3 days, shaking occasionally, filter, neutralize the filtrate, while warming gently, with Na_2CO_3, and filter again from the acid albumen thus precipitated. Then evaporate to about 200 c.c., filtering again before reaching this point if more albumen becomes separated. The reaction meanwhile must be kept neutral by adding HCl or Na_2CO_3, as the case should require. Now acidify the solution with acetic acid, saturate with NaCl, and filter. The filtrate contains peptones. Test for the same.

XII.

Clinical Analysis of the Stomach Contents.

For clinical examination the stomach contents should be filtered, or if too thick and difficult of filtration, they should be diluted with a definite amount of distilled water. In quantitative estimations the amount should be measured with a pipette and not a graduate.

1. Presence of Pepsin. — Take 10 c.c. of the stomach contents, place in a test-tube, and add a thin slice of hard-boiled egg or fibrin. Place in a brood oven, and allow to remain a few hours. If after 10 hours there is no diminution of the size of the egg, or if there is an odor of decomposition, then pepsin is absent. If the stomach contents are weakly acid or alkaline, add an equal amount of digestive HCl.

2. Presence of Rennet. — Take 5 c.c. neutralized stomach contents, and add to it an equal amount of cooked, neutral cow's-milk, which has been fully cooled after cooking. Place in a brood oven, and if rennet be present, after 20 or 30 minutes the casein of the milk will have separated out in flocculent masses.

3. Presence of Free HCl. — Use methyl-violet — tropæolin 00 — Günzburg's reagent or dimethyl-amido-azo-benzol.

4. Quantitative Estimation of Total Acidity. — Take 10 c.c. of the filtered stomach contents, add a few drops of rosolic acid until yellow, and titrate with a $\frac{1}{10}$ normal solution NaOH until a permanent rose-red color is obtained.

C a l c u l a t i o n. — 1 c.c. NaOH = .00365 grammes HCl.

5. Quantitative Estimation of Total HCl (free and combined).

Take 15 c.c. of the filtered stomach contents, add 1 gramme calcium carbonate, stir well, and filter. Take 10 c.c. of the filtrate, place in a small flask, and pass air through until the CO_2 is all driven off. Then add 5 c.c. calcium chloride, a few drops of rosolic acid, and titrate with $\frac{1}{10}$ normal NaOH as before. The difference between the acidity found before and that found now equals the total HCl (free and combined).

1 c.c. NaOH = .00365 grammes HCl.

The principle of this method is based upon the fact that the calcium carbonate combines with the free and combined HCl and the organic acids to form neutral calcium salts, while the acid phosphates are not affected; and by determining as in (4) the total acidity, and deducting from this the acidity due to the acid salts, the amount of total HCl is obtained.

6. Test for lactic acid by Uffelmann's reagent.

7. Acetic, Butyric, and Formic Acids. — Distil a portion of the stomach contents, and, if present, these acids will be found in the distillate.

(*a.*) **Test for Acetic Acid.** — Accurately neu-

tralize a portion of the distillate with NaOH and then add ferric chloride. Red color, if present.

(*b.*) **Tests for Butyric Acid.** — 1. Add ferric chloride. Red color, which disappears on heating. 2. Add conc. H_2SO_4, and warm. Characteristic odor of rancid butter.

(*c.*) **Test for Formic Acid.** — Add silver nitrate. White pp., which turns black in the dark.

8. Albumose Peptone. — Neutralize, while warming, with Na_2CO_3, filter, and test filtrate by biuret test.

9. Starch and Erythrodextrine. — Test with a solution of iodine in potassium iodide. They should not be present an hour after food is taken.

10. Resorptive Power of the Stomach. — Give a capsule containing 0.1 gramme potassium iodide, and test the saliva every few minutes with filter-paper moistened with starch solution until a blue color is obtained. It ought to be absorbed in from 8 to 15 minutes in a normal stomach.

11. Motor Power of the Stomach. — A capsule containing 1 gramme of salol is given, and separate portions of the urine, passed within intervals of half an hour, are tested by ferric chloride. The violet color, due to salicyluric

acid, is obtained, under normal conditions, in from 45 to 75 minutes.

Microscopic Examination.

Sarcinæ. — They occur in the form of peculiar colonies of cocci, arranged in squares, and strongly resembling cotton-bales. In normal conditions they are found only in small numbers.

Yeast-Fungi — have a characteristic oval form, arranged in buds. They are diagnostic of fermentative dyspepsia.

XIII.

The Bile.

Biliary Acids (Glycocholic and Taurocholic), Pettenkofer's Test. — Take a few c.c. of bile, add an equal amount of water and a few drops of cane-sugar solution. Then pour one-half the amount of conc. H_2SO_4 down the side of the test-tube, so that the latter shall underlie the bile. At the juncture of the bile and the acid there is found a purple-red zone. Then dip the test-tube into a glassful of water, and mix the two fluids carefully by shaking or swaying the tube to and fro (if the resulting

heat is developed too suddenly the test is spoiled), and we obtain a magnificent purple-red fluid.

After the tube is fully cooled, pour one-half into another tube containing alcohol, and the second half into a third tube containing glacial acetic acid.

(*a.*) The acetic acid solution shows in the spectroscope a line in green.

(*b.*) The alcoholic solution shows at first only the line in green, but the solution soon becomes brownish, and we find a line in blue.

The results of these tests show that we have present both glycocholic and taurocholic acids, as well as cholic acid, which forms the basis of both.

This test can be modified by diluting the bile tenfold, and taking a few drops of the dilute solution, and adding a trace of sugar and a few drops conc. H_2SO_4. Evaporate in an evaporating dish upon the water-bath, and a violet-red color appears upon the dish at the edge of the fluid. As soon as this appears cease evaporating.

Biliary Pigments (Bilirubin and Biliverdin), Gmelin's Test. — Dilute some of the bile ten times, take a portion in a test-tube, and pour down the side of the tube some impure

HNO_3 (nitric acid containing HNO_2), so that the acid shall underlie the bile. There is formed at the junction of the two fluids a play of colors, consisting of green, blue, violet, red, and reddish-yellow. The green must always be present. The play of colors in this test is the result of oxidation, the part nearest the acid being most intensely oxidized.

Huppert's Test. — Take some dilute bile solution, and add lime-water as long as a pp. is formed. Filter the pp. off (which consists of bilirubin lime), wash once with water, and place while still moist in a test-tube filled half full with alcohol which has been acidified with sulphuric acid. Heat to boiling in the water-bath. The fluid takes on a brilliant green or bluish-green color.

XIV.

Pancreatic Juice.

Trypsin. — Dissolve 1 gramme powdered extract of pancreas in 500 c.c. water, and add 5 c.c. of a saturated sodium carbonate solution. Place in a flask, and add the whites of two hard-boiled eggs finely chopped. Add a few drops

of chloroform, cork the flask tightly, and place in a brood oven for a few days, shaking occasionally. Now acidify carefully with acetic acid while warming, and filter. Shake a portion of the filtrate in a test-tube with bromine water. It should give a violet-red color. Now evaporate the filtrate to about 100 c.c. and allow to stand in a cool place until the next day. Separation of white crystals of tyrosin. Filter these off (preserving the filtrate) and examine under the microscope. Colorless, needle-shaped crystals of tyrosin. Dissolve some in water in test-tube, add a few drops of Millon's reagent, and heat to boiling. Rose-red to deep-red color.

Scherer's Test. — Evaporate a few of the tyrosin crystals to dryness on platinum foil with nitric acid. A yellow residue is obtained, which on adding NaOH develops a deep reddish-yellow color.

The filtrate from the above is to be evaporated still further upon the water-bath, until there is formed a scum upon the surface of the fluid. This scum is leucin. Examine under the microscope. Crystals of leucin, which consist of globules having a pearly lustre and radial lines. Easily soluble in HCl and alkalies.

Diastase. — Add 1 gramme pancreas powder

to 50 c.c. water, digest for two hours at 40° C., and filter. Take equal parts of the filtrate and starch clyster (made as for saliva) and digest for a short time in the brood oven. The starch solution becomes transparent and shows the presence of sugar by Trommer's test.

PART II.

THE URINE.

THE URINE.

I.

General Characteristics.

Color. — Normal { Pale. Normal. High.

Pathological { Blood. Bile. Chyle.

Accidental — due to drugs.

Odor. — Accidental (due to drugs). Urinous. Ammoniacal. Due to acetone.

Reaction. — Acid or alkaline. The alkali may be fixed or volatile. If fixed, it does not disappear on heating; if volatile, it disappears on heating.

Specific Gravity. — Normal, 1015–1025.

Amount. — Subject to great variation. Normal, 1,500 c.c.

Solids by Specific Gravity. — Multiply last two figures of specific gravity by 2.33, which gives the number of grammes of solids in 1,000 c.c.

II.

General Chemistry and Clinical Analysis.

Urobilin. — Take a little H_2SO_4 in a test-tube and pour on it, from a height, some urine. Purplish-brown color, if present.

If decreased — light color.

If normal — dark and transparent.

If increased — dark and opaque.

Indican (Stokvis' Test). — To $\frac{1}{3}$ test-tube of urine add an equal amount HCl and one or two drops calcium hypochlorite, and shake. Then add a few drops of chloroform and shake thoroughly. If indican is present, the chloroform will be colored blue. If normal, it will be about the color of litmus.

Albumen (Qualitative).

Serum Albumen. Nitric Acid Test. — To about $\frac{1}{2}$ wine-glassful of urine add with a pipette about $\frac{1}{5}$ the amount of conc. HNO_3, so that the acid shall underlie the urine. If present, there will be a distinct white zone at the point of contact, which is sharply defined and extends either way. If bile be present,

the albumen zone will be tinged green. In the nitric-acid test several other zones will be present, viz.:

1. A nitrate of urea zone, which always occurs in the nitric acid and extends downwards.

2. A zone of indican and urobilin, which is exactly below the albumen zone.

3. A zone due to urates, which appears above the nitric acid in the urine and extends upwards.

4. A mucin zone in the upper part of the urine.

Heat Test. — To some filtered urine in a test-tube add a drop of dilute acetic acid, and heat the upper part of the fluid. If present, there will be a cloudiness to a coagulation, which can be best seen by comparing it with the clear fluid below. This test, if properly performed, is a very delicate one.

Nucleo-Albumen. — Add a few drops of 10% acetic acid. If present, there will be a pp. soluble in excess of the acid.

Mucin. — Same as for nucleo-albumen, but *not* soluble in excess of acetic acid.

Globulin. — Dilute a small amount of urine with several times its bulk of water. If present, a pp. will be formed.

Albumose-peptone. — Remove the other albumens by 10 % acetic acid and heating; filter, and test filtrate by: (*a*) Biuret reaction. (*b*) Take 10 c.c. of above urine, add 1 c.c. HCl, and then precipitate with phospho-tungstic acid. Warm slightly, let pp. fall to the bottom of the tube, pour off the fluid above, and wash pp. with water. Dissolve pp. in NaOH and add a weak solution $CuSO_4$. Biuret reaction.

Albumen (Quantitative).

Esbach's Test. — Fill to the mark U with clear, filtered urine, and then add Esbach's reagent to the mark R. Let stand for 24 hours or shake down in a centrifuge. Every mark occupied by the pp. represents $\frac{1}{10}$ of 1 % of albumen, or 1 gramme to the liter.

Sugar (Qualitative).

Trommer's Test. — Take $\frac{1}{2}$ test-tube of urine, add an equal amount of NaOH, and then add $CuSO_4$ until it no longer dissolves. Heat the upper part, and if sugar be present there will be a reduction of the copper to the red suboxide.

Fehling's Test. — Take a few c.c. of Fehling's solution, dilute with about 4 times the amount of water, and heat to boiling. Then add a

little urine and heat once more near the upper part of the fluid. Reduction of copper, if sugar is present, to red suboxide.

Nylander's Test. — Add to the urine about $\frac{1}{5}$ the amount of Nylander's solution, and boil. Reduction of metallic bismuth if sugar is present.

Sugar (Quantitative).

Fehling's Method. — First ascertain the specific gravity.

If 1.030 or below, dilute 5 times, by taking 10 c.c. of urine and adding 40 c.c. water.

If above 1.030, dilute 10 times, by taking 5 c.c. urine and adding 45 c.c. water.

Place the urine in a burette. Take 10 c.c. Fehling's solution, place in a small flask, and add 40 c.c. water. Bring to a boiling point, and add the urine a little at a time, boiling between each addition. Continue this until the blue color has entirely disappeared.

Calculation. — 10 c.c. Fehling's solution = .050 grammes sugar; therefore the amount of mixed urine used in the titration, divided by its degree of dilution, contains exactly .050 grammes sugar. Calculate entire amount for 24 hours.

Fermentation Test. — Ascertain accurately the specific gravity of the urine. Then take 50

c.c. of the urine and add 2 grammes yeast. Place in a bottle which is closed tightly and through the cork of which is passed a reversed pipette. Place in a warm place and allow to stand for 24 hours. Again take the specific gravity after fully cooled, and the difference multiplied by 230 gives the percentage of sugar.

Note. — In all quantitative estimations albumen must be removed, and the urine measured with a pipette and not a graduate.

Urea (Qualitative).

To a few drops of urine on a microscopic slide add a drop of conc. HNO_3, and in a few minutes crystals of urea nitrate will have separated out. Examine with a microscope.

Quantitative (Liebig's Method).

Take 20 c.c. urine and add to it 10 c.c. baryta mixture. Then filter, take 15 c.c. of the filtrate, which should be clear, place in a beaker, and titrate with a standard solution of mercuric nitrate, until a drop, added to a saturated solution sodium carbonate, gives a yellow pp. of oxide of mercury.

Calculation. — 1 c.c. mercuric nitrate = .010 grammes urea. Gives amount of urea in 10

c.c. urine. Calculate entire amount for 24 hours.

Normal amount, 25 to 40 grammes in 24 hours.

Uric Acid (Qualitative).

Take 20 c.c. urine and add to it 2 c.c. conc. HCl. Set aside for 24 hours in a cool place. The uric acid which has separated out is collected on a filter and subjected to the murexid test.

Murexid Test. — Place the crystals in an evaporating dish, and dissolve them in a few drops of HNO_3 with the application of heat. Evaporate carefully to dryness, when a yellowish-red spot will be found to remain. Cool, and add a drop of ammonia, when a beautiful purplish-red color will develop, owing to the formation of ammonium purpurate (murexid).

Quantitative (Hopkins' Method).

Take 100 c.c. urine and add 30 grammes of finely powdered ammonium chloride. The mixture is then allowed to stand for two hours, and is stirred from time to time. Then filter and wash the pp. two or three times with a saturated solution $(NH_4)Cl$. Wash the pp. from the filter, with a little boiling water, into an

evaporating dish, and add 5 c.c. HCl. Evaporate the solution until crystals of uric acid begin to separate out. These are collected on a filter, the filter-paper with the adherent pp. is placed in a beaker, and the crystals are dissolved in as small an amount of a 30% solution of sodium carbonate as possible, with the aid of heat. Cool, dilute with water to 100 c.c., add 20 c.c. conc. H_2SO^4, and warm. Titrate, while warm, with a $\frac{1}{20}$ normal solution potassium permanganate, until the rose-red color no longer disappears on stirring.

Calculation. — 1 c.c. potassium permanganate = 3.61 milligrammes uric acid. Gives amount of uric acid in 100 c.c. urine. Calculate entire amount for 24 hours. Normal amount eliminated in 24 hours, about 1 gramme.

Chlorides (Qualitative).

To a few c.c. urine which has been acidified with a few drops HNO_3 add a few drops $AgNO_3$. White pp. of silver chloride.

Quantitative (Mohr's Method).

Take 10 c.c. urine, acidify with a few drops HNO_3, and dilute with distilled water to 50 c.c. Then add very carefully sodium carbo-

nate in substance until just neutral. Then add a few drops yellow K_2CrO_4, which gives the solution a yellow color, and titrate with a standard solution of silver nitrate until a distinct orange tinge is obtained after brisk stirring.

Calculation. — 1 c.c. $AgNO_3$ = .010 gramme chlorides. Gives amount of chlorides in 10 c.c. urine. Calculate entire amount for 24 hours.

Normal amount eliminated in 24 hours, 11–15 grammes.

Sulphates.

To a few c.c. of urine add a few drops of solution of barium chloride and HCl. A white pp. of barium sulphate.

Phosphates (Qualitative).

Total Phosphates. — A few c.c. of urine are acidified with a few drops of acetic acid and then treated with a solution of ferric chloride. A yellowish-white pp. of ferric phosphate.

Earthy Phosphates. — To a few c.c. of urine add ammonia in excess. A flocculent pp. indicates their presence.

Alkaline Phosphates.— Precipitate the earthy phosphates with NH_4OH, filter, and to the clear filtrate add magnesium sulphate solution. A heavy white pp.

Quantitative Estimation of Phosphoric Acid.

Take 50 c.c. urine and add 5 c.c. of sodium acetate solution. Heat the mixture to boiling, and titrate with a standard solution of uranyl nitrate, until a drop, placed in a solution of potassium ferrocyanide, gives rise to a mahogany-brown pp.

Calculation. — 1 c.c. uranyl nitrate = .005 gramme P_2O_5. Gives amount of phosphates in 50 c.c. urine. Calculate entire amount for 24 hours.

Normal amount eliminated in 24 hours, 1–2 grammes.

Bile Pigments.

Gmelin's or Huppert's Test. — Green must always be present.

Blood Coloring Matters.

Determine with a spectroscope which coloring matter is present.

Guaiac Test. — To a few c.c. of urine add a few drops of freshly prepared tincture guaiac and a little old oil of turpentine. Shake thoroughly, and if blood is present the mixture becomes blue.

Kreatinin.

A few c.c. of urine are treated drop by drop with a very dilute solution of sodium nitroprusside, and then drop by drop with a solution NaOH. In the presence of kreatinin a ruby-red color develops, which is best seen in the lower portion of the tube. Then add a few drops glacial acetic acid and warm, and the color changes to green.

Acetone.

1. Same test as for kreatinin, except that on the addition of acetic acid a purple instead of a green color develops. This takes place without warming.

2. A few c.c. of urine are treated with a few drops of iodine in potassium iodide solution and NaOH. Warm, and a pp. of iodoform occurs, which may be recognized by its odor. If these reactions for acetone fail, take about 1 liter of urine, add a few drops H_2SO_4 and distil about $\frac{1}{2}$, and then redistil about 10 c.c. As acetone is very volatile, if any is present it will be found in the 10 c.c.

Aceto-Acetic Acid.

Add ferric chloride solution. There is a pp. of ferric phosphate formed, which is allowed to

settle. On the further addition of ferric chloride, a purple color is formed if the reaction is positive.

Ehrlich's Diazo-Reaction.

To a few c.c. of urine add an equal amount of sulphanilic acid and a few drops of sodium nitrite. Shake thoroughly, and then allow 1 or 2 c.c. of ammonia to run down the side of the tube so as to overlie the fluid below. If the reaction is positive, a carmine-red ring develops at the junction of the two fluids. This reaction takes place in febrile urines, and is due to a peculiar chromogen. The test was formerly thought to be especially diagnostic of typhoid fever.

III.

Urinary Sediment.[1]

Unorganized Sediments.

Uric Acid.—Whetstone shape. Almost always colored yellow or brown. Forms the brick-dust sediment. Soluble in NaOH.

[1] To obtain a urinary sediment, allow the urine to stand in a conical glass over night, or, what is better, throw it down with the centrifuge, and with a pipette transfer a small portion to a slide, cover with a cover-glass, and examine with a high power. Do not use a condenser.

Urates.—Sodium and potassium are amorphous. Ammonium, dumb-bell shape, or round, or sheaf-shaped.

Oxalate of Calcium.—Envelope, dumb-bell or oval shape. Insoluble in acetic acid.

Phosphates.—Coffin-lid shape. Soluble in acetic acid.

Cystin is very rare. Small, colorless, hexagonal crystals, soluble in ammonia.

Hippuric acid (in long needles), leucin (spherules with concentric striations), and tyrosin (in fine needles) are not common.

Organized Sediments.

Blood.—Normal and abnormal shapes of corpuscles: that is, round or shrivelled (crenated).

Leucocytes.—Resemble distended red blood corpuscles.

Pus Corpuscles.—Are round, with nuclei and granules. On the addition of acetic acid the granular matter is destroyed and the nuclei are brought out very clearly.

Epithelial Cells.—Are either normal, granular, or fatty. These three conditions mean simply the age of the process.

Renal (from convoluted tubules)—are slightly polygonal, with nucleus or with granules, nucleus persisting.

Renal (from straight tubules) — are less polygonal and more circular.

Pelvic — battledore shape, if superficial; round, if from the deeper layers.

Ureter — spindle-shaped cells.

Bladder — from fundus polygonal; from neck round.

Vaginal — large and very faint, frequently overlapping.

Urethral — spindle-shaped.

Prostate — resemble pelvic cells (deep).

Fat may be found as free fat, or dotting epithelial cells, or as so-called compound granular cells.

Casts.

Hyaline — faint, with both ends rounded.

Fibrinous — contains a substance of greater density. Varies from hyaline only in intensity.

Blood — cast covered with blood corpuscles and leucocytes.

Epithelial — cast covered with renal epithelial cells.

Granular — cast covered with granules.

Fatty — cast covered with fat globules.

Waxy — are very distinct, intense, and homogeneous. Slightly yellow.

Fungi and Bacteria.

Micrococcus Ureæ. Produces ammoniacal fermentation, converting urea into ammonia.

Bacterim Termo. Rod-like shape.

Pencillium Glaucum. Grows in chains. Individuals are oval-shaped, with a little depression at each end. Occurs only in acid urines.

Yeast Fungi. Found only in diabetic urines, or in urines containing sugar. Ovals with depressed centres.

Sarcinæ. Analogous to the sarcinæ found in the stomach.

Tubercle Bacilli. Found in tuberculosis of the genito-urinary tract. Do not confound with the smegma bacillus, which morphologically resembles the tubercle bacillus.

Spermatozoa.

PART III.

TOXICOLOGY.

I.

Acids.

If unmixed with organic matter, concentrate if too dilute.

Sulphuric Acid.

A. First determine whether a free acid or a sulphate is present, by methyl-violet or dimethyl-amido-azo-benzol.

1. To a small portion of the fluid add acetate of lead solution. White pp.

2. To another portion add barium chloride solution. Heavy white pp.

3. To another portion add calcium chloride solution. Insoluble white pp.

B. If mixed with organic matter (stomach contents, etc.), concentrate to a syrup, extract with successive portions of alcohol, and drive off the alcohol by warming on the water bath. A watery solution is left, and with it perform tests 1, 2, 3.

Nitric Acid.

A. If unmixed, test for free acid, to distinguish from nitrates.

1. Boil a small portion with a little albumen (white woolen thread or peptone) — yellow color. Cool, and add NaOH — orange color. (Xanthoproteic reaction.)

2. Add to a small portion in a test-tube a piece of copper and some concentrated sulphuric acid, and heat. Red fumes.

3. Add to a portion some sulphuric acid, and pour carefully down the side of the tube some ferrous sulphate solution. Brown zone at point of contact.

B. If mixed with organic matter, concentrate, cool, extract rapidly with alcohol, neutralize with potash, filter, and drive off the alcohol. Use tests 2 and 3. Reacts equally well with nitrates.

Hydrochloric Acid.

A. If unmixed, test for free acid.

1. Add silver nitrate. White pp. soluble in ammonia, which darkens on exposure to light.

2. Add lead acetate solution. Heavy, white, crystalline pp.

3. Add mercurous nitrate solution. Fine white pp.

B. If mixed with organic matter, distil, and test distillate.

Oxalic Acid.

Does not react to tests for free mineral acids.

A. If unmixed:

1. Add calcium chloride or calcium sulphate solution. Fine powdery pp. of calcium oxalate. Examine with the microscope.

2. Add silver nitrate solution. White pp. of silver oxalate, soluble in ammonia, and with some difficulty in nitric acid. Silver chloride is insoluble in nitric acid.

3. Add baryta water. White pp.

B. If mixed with organic matter, acidulate with HCl: evaporate to dryness, extract hot (by boiling) with alcohol: drive off the alcohol and dissolve residue in water. Apply all the tests.

Carbolic Acid.

Does not react to tests for free mineral acids.

A. If unmixed:

1. Add ferric chloride solution. Deep amethyst-blue color.

2. Add to a small portion ¼ its volume of ammonia, a few drops calcium hypochlorite solution, and warm gently, but not to boiling. Blue to green color.

3. Add a few drops Millon's reagent and heat to boiling. Intense dark-red color.

4. Add bromine water. A gelatinous pp. of tribromophenol, soluble in excess of carbolic acid.

B. If mixed with organic matter, acidify strongly with HCl and distil. Apply all the tests.

Hydrocyanic Acid (Potassium Cyanide).

A. If unmixed with organic matter:

1. Add a few drops ferrous sulphate solution, then a few drops of ferric chloride, and acidify strongly with HCl. Blue pp. of Berlin blue.

2. Add some ammonium sulphide, evaporate to dryness upon the water bath, acidify with HCl, and add a couple drops of ferric chloride. Blood-red color from sulphocyanide of iron.

3. Add a few drops of picric acid, and warm. Blood-red color from isopurpurate of potash.

4. Add silver nitrate. White pp. of silver cyanide.

B. If mixed with organic matter:

1. Place a piece of filter-paper moistened with guaiac mixture in the cork of the flask, and warm. Filter-paper becomes blue.

2. Acidify with tartaric acid and distil into a weak potash solution. The delivery tube must pass below the surface of the solution, to prevent escape into the air. With the distillate perform the tests.

II.

Metallic Poisons.

Arsenic (Arsenious Acid).

A. If unmixed with organic matter:

1. Place a small granule of white arsenic in the closed end of a reduction tube, and above this place a small splinter of charcoal. First heat where the charcoal is, then the closed end of the tube, and just above the charcoal there is formed a mirror of metallic arsenic. Now remove the charcoal and heat the mirror, and a sublimate of white arsenic is formed above it. Examine with a microscope. Octahedral crystals.

2. Slightly acidify some of the solution with HCl, and pass H_2S through it. Yellow sulphide of arsenic is formed, which is soluble in ammonia.

3. Add ammonia to some of the solution, drive off the excess by warming on the water bath, and divide into 2 parts. To:

(*a.*) Add silver nitrate, and a yellow pp. of silver arsenate is formed. To:

(*b.*) Add copper sulphate. A pale green pp. of arsenate of copper (Scheel's green).

B. If mixed with organic matter:

Reinsch Test. — Strongly acidulate the solution with HCl, place in it a piece of bright copper tinsel, and warm 10 to 15 minutes over the water bath. The copper, which has become covered with a grayish-white layer, is now to be removed, washed with water, alcohol, and ether, and thoroughly dried. Place in a small reduction tube and heat gently. Sublimate of arsenious acid formed in the cool part of the tube. Examine with the microscope. Octahedral crystals. This method, although not as delicate as the Marsh test, possesses the advantage that it can be applied in the presence of organic matter.

Antimony (Tartar Emetic).

A. If unmixed:

1. Add to some of the solution $\frac{1}{10}$ its volume of HCl and place in the solution a piece of tin. Black deposit of antimony upon the tin in the cold.

2. Acidulate some of the solution with HCl and pass H_2S through it. Orange-red pp. of sulphide of antimony.

3. Add nitric acid drop by drop to some of the solution. White pp. of subnitrate of antimony, soluble in excess of nitric acid.

B. If mixed with organic matter:

Fresenius-Babo Method. — Acidify strongly with HCl ($\frac{1}{4}$ its volume), and add, while warming, chlorate of potash, until the solution is yellow. Drive off the free chlorine, filter, dilute largely with water, and pass H_2S through. Orange-red pp. of sulphide of antimony. As arsenic may give a somewhat similar pp. and organic matter still be present, filter out the pp. dry, and burn with saltpeter mixture. After cooling, dissolve the residue, with warming, in dilute HCl (1 : 2), and to the clear solution apply the individual tests.

Mercury (Corrosive Sublimate).

A. If unmixed:

1. Pass H_2S through a small portion, and there is formed a pp. which is at first yellow and finally black.

2. Add NaOH to some of the solution. Yellow pp. of mercuric oxide.

3. Add $(NH_4)OH$. White pp. of amido-mercuric chloride, soluble in HCl.

4. Add subchloride of tin, and there is formed a white pp. of subchloride of mercury or a gray one of mercury.

5. Add potassium iodide. Scarlet pp. of mercuric iodide.

B. If mixed with organic matter:

Acidify with HCl, insert a piece of bright copper tinsel, and warm over the water-bath. Remove the copper, wash, dry, and heat in small ignition tube. Sublimate of mercury formed in the cool part of the tube. Place a small granule of iodine above the sublimate in the tube, and heat the sublimate once more. Bright red zone of iodide of mercury. Examine with the microscope. Diamond-shaped crystals.

III.

Metallic Poisons (Continued).

Copper (Copper Sulphate).

A. In pure solution:

1. Pass H_2S through the solution. Black sulphide of copper.

2. Add some potassium ferrocyanide. Brownish-red pp.

3. Add ammonia to some of the solution. Blue pp., soluble in excess of the ammonia, giving a deep-blue fluid.

B. If mixed with organic matter:

1. Accidulate with HCl and insert a piece

of steel. Bright-red layer of copper upon the steel.

2. Destroy organic matter by HCl and $KClO_3$ (Fresenius-Babo), filter, dilute filtrate, and pass H_2S through. Black pp., which can be further verified by the above tests.

Lead (Lead Acetate).

A. In pure solution:

1. Add sulphuric acid. White pp. of lead sulphate.

2. Add HCl. White pp. of lead chloride, insoluble in ammonia. (Distinction from silver chloride.)

3. Add potassium ferrocyanide. White pp. of ferrocyanide of lead.

4. Add potassium iodide. Yellow pp. of lead iodide.

B. If mixed with organic matter:

Destroy organic matter with HCl and $KClO_3$, filter while hot, and, upon cooling and diluting, white crystalline lead chloride may be deposited. This can be redissolved in hot water and the tests applied. If the chloride is not deposited, pass H_2S through the solution, filter off the black lead sulphide, and dissolve in warm nitric acid. Verify by the tests.

Phosphorus.

1. Fasten a piece of filter-paper, moistened with silver nitrate solution, to the cork of the flask containing the mixture, and warm slightly (by placing the flask in warm water). Filter-paper becomes blackened, due to the formation of silver phosphide. This must take place in the dark, as silver nitrate is blackened by light alone. It is also best to place a second piece of filter-paper in the cork, moistened with lead acetate, to exclude hydrogen sulphide, which would also blacken silver nitrate.

2. Acidify a portion with sulphuric acid, and distil into a weak solution of silver nitrate. If examined in the dark, the vapor is phosphorescent in the cooler, and the silver is precipitated as a black phosphide of silver.

Nitro-Benzol.

A. If unmixed:

1. Mix some of the solution with alcohol, add NaOH and some sulphur or the sulphide of an alkali. Red color, if present.

2. Distil a portion, dissolve the oily drops of the distillate in alcohol, and treat with zinc and dilute H_2SO_4. From this aniline is formed. Render alkaline with NaOH, shake with ether, allow the ether to evaporate, and treat the resi-

due with chloride of lime solution. Violet color.

3. Distil a portion, add to distillate a few drops KOH, boil, and then add carbolic acid. Evaporate to a ring of dryness, and touch the ring with calcium hypochlorite solution. Green color, if present.

B. If mixed with organic matter, distil, and apply all the tests.

For the detection and the isolation of the metallic poisons when mixed with organic matter, the ideal method is that of Fresenius-Babo; that is, the destruction of organic matter with hydrochloric acid and potassium chlorate, as given above. In the metals under consideration it can be applied to antimony, copper, lead, arsensic, and mercury. As the two latter are volatile, care must be taken against their possible loss, by conducting the operation in a flask containing a cork with a long tube, instead of in an evaporating dish, as with antimony, copper, and lead. In the case of arsenic, the latter procedure is not absolutely necessary, provided care be taken to add the potassium chlorate before heating.

IV.

Alkaloids.

If unmixed with organic matter:

Morphine.

1. Take 1 or 2 c.c. of the solution in a test-tube, acidify with sulphuric acid, add a couple drops of chloroform, a small granule of iodic acid, and shake thoroughly. Chloroform becomes colored red, showing the presence of morphine.

2. Take a granule of the substance (or if in pure solution, evaporate to dryness on the water-bath), add dilute nitric and sulphuric acids (a drop of each only), warm, and allow to cool. Blood-red color.

3. Dissolve a granule in a couple drops of nitric acid. Orange-red color.

4. Add to a granule a drop of Froehde's reagent. Colored violet, green, blue-green, and yellow.

Strychnine.

1. Dissolve a granule or two in concentrated sulphuric acid in a porcelain dish, and then add a small granule of red chromate of

potash. Colored blue, violet, and finally cherry-red.

2. Add a drop of dilute nitric acid to a granule, in an evaporating dish, and warm carefully. Now add to the warm solution a small crystal of chlorate of potash. Intense scarlet color, which changes to brown upon the addition of 1 or 2 drops of ammonia, with the formation of a brown pp.

Veratrine.

1. Warm with hydrochloric acid. Beautiful red color.

2. Add to some of the dry alkaloid some cane-sugar, and moisten with concentrated sulphuric acid. Yellow, blue, and finally a violet color.

3. With Froehde's reagent. Yellow, then cherry-red.

Atropine.

1. Dissolve a portion of the solid in fuming nitric acid, evaporate to dryness upon the water bath, and moisten, after cooling, with a 10% alcoholic KOH solution. Violet, then cherry-red color.

2. Heat a portion of the dry alkaloid in a small closed tube, or in an evaporating dish,

over a free flame, until white fumes begin to rise. Odor of violets.

Cocaine.

1. Add 5% chromic acid solution to a portion, and there is formed a pp. which at first becomes redissolved, but after a time returns as a crystalline orange-colored pp. of cocaine chromate. This reaction takes place more quickly upon the addition of a few drops of conc. HCl.

2. Add 5% chromic acid solution, and warm. Green color, with fumes of benzoic acid.

3. Add drop by drop 1% potassium permanganate solution. Small violet crystals of permanganate of cocaine.

4. Touch a small portion of a dilute solution to the tongue, and there is caused a numbness lasting for some time.

If the alkaloid is mixed with organic matter, in order to isolate it it is necessary to resort to the method of Stas-Otto.

The Method of Stas-Otto.

1. Evaporate upon the water-bath to a syrup.

2. Transfer to a flask containing a cork with

a long tube, add twice as much of a 1% alcoholic tartaric acid solution, and warm upon the water-bath from 30 minutes to an hour.

3. Cool, filter, concentrate the filtrate until the alcohol is driven off, and filter once more through a filter moistened with water.

4. Evaporate the filtrate once more to a syrup.

5. Add alcohol again, stirring until all is separated which is not soluble.

6. Filter once more, drive off the alcohol by evaporation, dissolve the residue in water, render alkaline by NaOH, and shake in a separating funnel with ether. Strychnine, veratrine, atropine, and cocaine are found in the ether, which is allowed to evaporate spontaneously in an evaporating dish, and the residue verified by the above tests.

7. Free from ether by warming upon the water-bath, add ammonium chloride until ammoniacal, and allow to stand over night. Then warm, and shake with amyl alcohol. Morphine found in the amyl alcohol. Allow to evaporate, and verify by the above tests.

V.

Scheme for the Detection of an Unknown Poison.

A fresh specimen is to be taken for each test.

1. Add methyl-violet or dimethyl-amido-azo-benzol.

Shows free mineral acids:

Sulphuric.

Nitric.

Hydrochloric (volatile).

2. Add ferric chloride. Blue color shows carbolic acid (volatile).

3. Take a portion in 3 test-tubes and acidify with HCl. Place in:

(*a.*) A piece of bright copper tinsel, and warm. Deposit shows arsenic, mercury, or antimony.

(*b.*) A piece of bright tin. Black deposit in the cold shows antimony.

(*c.*) A piece of bright steel. Red deposit shows copper.

4. Add potassium iodide. Yellow pp. Or moisten a piece of filter-paper with the solu-

tion and place in a stream of H_2S. Black shining deposit shows

Lead.

5. Take a portion in 2 test-tubes. Place in:

(*a.*) A strip of filter-paper moistened with guaiac mixture. Warm gently. Blue color shows hydrocyanic acid.

(*b.*) A strip of filter-paper moistened with silver nitrate. Warm gently. Black deposit in the dark shows phosphorus.

6. Distil into water. In distillate is found:

Hydrochloric acid.
Carbolic acid.
Hydrocyanic acid.
Phosphorus.
Nitro-benzol.

7. Extract with alcohol:

Sulphuric acid.
Nitric acid.
Oxalic acid.

8. Extract with alcohol and tartaric acid (Stas-Otto):

Morphine.
Strychnine.
Atropine.
Veratrine.
Cocaine.

9. Destroy organic matter with hydrochloric acid and potassium chlorate (Fresenius-Babo):

Arsenic.
Antimony.
Mercury.
Lead.
Copper.

APPENDIX.

Reagents.

Alcohol. — The alcohol must be colorless and leave no residue upon evaporation.

Sulphuric Ether.

Ammonia. — Liq. ammon. caust.

Ammonium Carbonate.

Alkaline Barium Solution (Baryta mixture). — Equal parts barium water and barium chloride solution.

Barium Water. — 1 part caustic barium.
15 parts water.

Dissolved warm, cooled, and filtered.

Barium Nitrate. — 1 : 12.

Barium Chloride. — 1 : 10.

Barium Carbonate. — Precipitated out of a barium chloride solution by ammonium carbonate and well washed.

Lead Acetate. — 1 : 10.

Subacetate of Lead Solution. — (Liq. plumbi subacetatis U.S.P.)

Bromine Water. — Water treated with an excess of bromine and cleared by allowing to stand, and decanting.

Ammonium Chloride. — 1 : 10.

Calcium Chloride. — 1 : 10.

Sodium Chloride. — Cold saturated solution.

Alcoholic Zinc Chloride. — Thick syrupy watery solution diluted with alcohol until the spec. grav. is 1.2.

Cochineal Tincture. — 5 grammes cochineal with 150 c.c. alcohol and 100 c.c. water. Allow to stand some days at room temperature, pour off, and filter.

Acetic Acid. — 30%.

Ferric Chloride. — 3 : 100.

Potassium Ferrocyanide. — 1 : 10.

Yellow Chromate of Potash (K_2CrO_4). — 1 : 20.

Potassium Nitrate. — Must be free from chlorides.

Potassium Hydrate. — 1 part potassium hydrate.
2 parts water.

Copper Sulphate. — 1 : 10.

Magnesium Sulphate. — Cold saturated solution.

Magnesium Mixture. — 1 part crystallized $MgSO_4$.
2 parts ammonium chloride.
4 parts ammonia.
8 parts water.

Millon's Reagent. — 1 part mercury with 2

parts nitric acid of spec. grav. 1.4. Gently warmed until the mercury is fully dissolved. One part of this solution is treated with 2 parts water.

Ammonium Molybdate. — 50 grammes of ammonium molybdate are dissolved in 200 grammes of ammonia. The solution is poured into 750 c.c. nitric acid of spec. grav. 1.2; allowed to stand some days in a warm place, and decanted.

Sodium Hydrate. — Spec. grav. 1.17.

Sodium Carbonate. — Saturated solution.

Sodium Phosphate (Na_2HPO_4). — 1 : 10.

Nessler's Reagent. 50 grammes potassium iodide dissolved in an equal amount of water, and as much mercuric chloride added while warming until the mercury is no longer dissolved. Then add a solution of 150 grammes KOH in 300 c.c. water, fill to a liter, add 5 c.c. more mercuric chloride solution, and let stand.

Nylander's Solution. — 4 grammes tartarate of sodium and potassium.
2 grammes bismuth subnitrate.
10 grammes NaOH in 90 c.c. water.

To be kept in a dark bottle.

Ammonium Oxalate. — 1 : 25.

Mercuric Chloride. — 1 : 20.

Phospho-Tungstic Acid. — 1 : 20, acidified with HCl.

Potassium Sulphocyanide. — 1 : 20.

Rosolic Acid. — 1 : 100 alcohol.

Hydrochloric Acid. — 1.124 spec. grav.

Nitric Acid. — Must be colorless; free from nitrous acid and HCl. Spec. grav. 1.2.

Sulphuric Acid. — 200 grammes conc. H_2SO_4 to 1 liter.

Silver Nitrate. — 1 : 30.

Tartaric Acid. — Crystals or powder.

Lactic Acid Solution. — 0.8 gramme : 100 c.c. water.

Digestive HCl Solution. — 10 c.c. 25% HCl to 1 liter water.

Sol. Methyl-Violet. — 0.5 : 1,000.

Sol. Tropæolin OO. — 0.25 : 1,000.

Günzburg's Reagent. — 1 gramme vanillin.
2 grammes phloroglucin.
100 c.c. alcohol.

Artificial Gastric Juice. — 0.5 gramme pepsin dissolved in 500 c.c. digestive HCl solution.

Fröhde's Reagent. — 0.1 gramme sodium molybdate dissolved in 10 c.c. conc. H_2SO_4.

Pacini's Fluid. — Mercuric chloride, 2 grammes.
Sodium chloride, 4 grammes.
Glycerine, 26 grammes.
Distilled water, 226 grammes.

Guaiac Mixture. — 0.5 gramme gum guaiac dissolved in 10 c.c. alcohol, to which are added a few drops weak copper sulphate solution (1 : 2,000). The mixture must be kept tightly corked.

Saltpeter Mixture. — 3 parts KNO_3.
1 part Na_2CO_3.

Dimethyl-amido-azo-benzol. — 0.5 gramme to 100 c.c. alcohol.

Sub-chloride of Tin. — Metallic tin dissolved in excess in HCl.

Tannic Acid. — 1 : 100.

Indigo Carmine. — Solution.

Alpha-Naphthol. — 15% solution in alcohol.

Iodine in Potassium Iodide (IKI sol.). —
Iodine, 1 gramme.
Potassium iodide, 2 grammes.
Water, 300 c.c.

Picric Acid. — Saturated solution.

Sodium Acetate Solution. — 100 grammes acetate of sodium dissolved in distilled water, and 100 c.c. of a 30% solution of acetic acid, the whole being diluted to a liter.

Sulphanilic Acid. — Sulphanilic acid, 2 grammes.
HCl, 50 c.c.
Distilled water, 1,000 c.c.

Diazo solution No. 1.

Sodium Nitrite. — 0.5% solution.

Diazo solution No. 2.

Standard Solutions.

$\frac{N}{25}$ **Solution Tartaric Acid** (for alkalinity of blood). — Contains 3 grammes tartaric acid to 1 liter distilled water.

1 c.c. = .0016 gramme NaOH.

Standardized with a $\frac{N}{10}$ solution NaOH.

$\frac{N}{10}$ **Solution NaOH** (for acidity of gastric juice). — Contains 4 grammes NaOH to 1 liter distilled water.

1 c.c. = .00365 gramme HCl.

Standardized with $\frac{N}{10}$ solution H_2SO_4.

Esbach's Reagent (for quantitative albumen). — 10 grammes picric acid.
20 grammes citric acid.
Dissolved in 1,000 c.c. distilled water.

Fehling's Solution (for quantitative sugar). — 34.64 grammes of pure crystallized copper sulphate, dissolved in about 200 c.c. distilled water.

173 grammes potassium and sodium tartarate.

125 grammes NaOH.

Both dissolved in 500 c.c. distilled water.

These two solutions are mixed together, and diluted with distilled water to 1 liter.

10 c.c. = .050 gramme glucose.

Standardized with a solution of glucose, 5 grammes to 1 liter distilled water.

Standard Solution Mercuric Nitrate (for quantitative urea). — 71.48 grammes pure metallic mercury, dissolved with warming in pure conc. HNO_3, and diluted to 1 liter with distilled water.

1 c.c. = .010 gramme urea.

Standardized with a solution of urea, 2 grammes to 100 c.c. distilled water.

$\frac{N}{20}$ **Solution Potassium Permanganate** (for quantitative uric acid). — 1.578 grammes pure crystals potassium permanganate to 1 liter distilled water.

1 c.c. = 3.61 milligrammes uric acid.

Standardized with $\frac{N}{20}$ solution oxalic acid.

Standard Solution Silver Nitrate (for quantitative chlorides). — 29.06 grammes pure silver nitrate to 1 liter distilled water.

1 c.c. = .010 gramme NaCl.

Standardized with a solution NaCl, 10 grammes to 1 liter distilled water.

Standard Solution Uranyl Nitrate (for quantitative phosphoric acid). — 33 grammes uranium oxide dissolved in pure HNO_3 and diluted to 1 liter.

1 c.c. = .005 gramme P_2O_5.

Standardized with 10.085 grammes Na_2HPO_4 to 1 liter distilled water.

Analysis of Blood.

Date. Name.

Qualitative Tests.

Oxyhæmoglobin. Methæmoglobin.
Carbon-monoxide-hæmoglobin. Hæmatin.
Hæmin crystals.
Sulpho-hæmoglobin.

Quantitative Tests.

Specific gravity. Alkalinity.
Hæmoglobin. (Fleischl's Hæmometer.)
Number of red corpuscles. (By Thoma-Zeiss method and Hæmatokrit.)
Number of white corpuscles.
Relation of white to red.

Diagnosis.

Remarks.

Analysis of Gastric Contents.

Date. Name.

Period after test meal.

Qualitative Tests.

Free HCl.	Pepsin.	Acetic acid.
Lactic acid.	Amylaceous matter.	Butyric acid.
Rennet.	Albumose-peptone.	Formic acid.

Quantitative Tests.

Total acidity.

Total HCl (free and combined).

Microscopic Analysis.

Sarcinæ.

Yeast fungi.

Diagnosis.

Remarks.